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Characterization of galactomannans isolated from legume endosperms of Caesalpinioideae and Faboideae subfamilies by multidetection aqueous SEC

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ABSTRACT

Galactomannans isolated from legume seed endosperms, including those of commercial interest, have been characterized by multidetection aqueous SEC. Galactomannans derived from seeds of the Faboideae subfamily had substantially higher M_w than those from Caesalpinioideae seeds ($M_{w,Fab}$ = 2.4–3.1 × 10^6 g/mol, $M_{w,Caes.}$ = 0.86–2.1 × 10^6 g/mol) and within the latter botanical subfamily, an apparent correlation between M_w and the degree of galactose substitution DG was found. The molar mass distributions were unimodal and differed primarily by a scale factor, with distributional widths narrower than a true Flory 'most-probable distribution'; good fits to Schulz–Zimm model were obtained. Across subfamilies no differences were found in the exponents of $[\eta]$–M and R_v–M relationships (0.61 ± 0.02, 0.54 ± 0.01, respectively), the Flory chain stiffness ratio (C_∞ = 20 ± 1 (BSF analysis)), or the persistence length (L_p = 5.5 ± 0.2 nm) obtained from SEC fraction data. However, it was found that prefactors in the $[\eta]$–M and R_v–M relationships as well as the unperturbed parameter K_Θ decrease in proportion to DG and therefore chain density. Generalized relationships incorporating galactose-dependent prefactors were therefore developed to model SEC fraction data of native galactomannans ($[\eta]_{GM} = (1800 \pm 200) \times M_o^{-1.61} \times M^{0.61\pm0.02}$, $R_{v,GM} = 0.63 \pm 0.05 \times M_o^{-0.54} \times M^{0.54\pm0.01}$) as well as lower-$M$ fractions obtained by ultrasonication ($[\eta]_{GM} = (730 \pm 100) \times M_o^{-1.71} \times M_w^{0.71\pm0.02}$, $R_{v,GM} = 0.49 \pm 0.05 \times M_o^{-0.57} \times M_w^{0.57\pm0.01}$, $M \approx 1 \times 10^5$-native). As a consequence of this dependence and the observed patterns in molar mass variation, $[\eta]$ varies within a narrow range for galactomannans as a whole despite substantial M_w differences.

1. Introduction

Galactomannans are high molar mass, water-soluble nonionic polysaccharides extracted in high yield from the endosperm tissues of many legume seeds, where they possess energy-reserve and hydration functions. Milled endosperm powders of guar (*Cyamopsis tetragonoloba*), carob (or locust bean) (*Ceratonia siliqua*), and fenugreek (*Trigonella foenum-graecum*) seeds are currently exploited on an industrial scale, and within the food industry are used as mass-efficient aqueous thickeners, co-gellants, and nutritional supplements (Gidley & Reid, 1995).

The 'classical' structure of legume galactomannans refers to a linear mannan backbone, comprising β-D-mannose units linked at carbons 1 and 4 through a flexible glycosidic bond, variably substituted at carbon 6 with α-D-galactose units. In aqueous solution, galactomannans adopt random-coil conformations with very large molecular dimensions and consequently are mass-efficient aqueous viscosifiers. The average degree of galactose substitution DG, as well as its substitution pattern varies according to the specific plant source, and applications have evolved to exploit these differences. In aqueous solution low-DG galactomannans such as carob interact strongly through galactose-depleted chain segments with compatible helices (e.g., xanthan, k-carrageenan) or by self-interaction, producing network-type gels that are useful texturants in many food products. High DG galactomannans on the other hand do not interact strongly with other polysaccharides and are used primarily as mass-efficient thickeners. Average DG has been found in fact to vary within a limited range depending on the botanical origin, with low-DG galactomannans confined primarily to Caesalpinioideae legume subfamily, and high-DG galactomannans to the Faboideae subfamily, based on a frequency analysis (Buckeridge, Panegassi, Rocha, & Dietrich, 1995). Further information on the details of the repeat unit structure, composition, and structure–property relations of galactomannans are documented in several reviews (Gidley & Reid, 1995; Shcherbukhin & Anulov, 1999; Srivastava & Kapoor, 2005).

Despite the appeal of these endosperm powders as robust thickeners and gellants, it has not been trivial to achieve reliable measure of the 'native' molecular weight of galactomannans, as

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found within the viable seed's endosperm. A major problem is the reliance on commercially available products such guar gum or locust bean gum, which are manufactured through a series of potentially harsh thermal and mechanical treatments (e.g., acid or flame peeling to extract endosperms, attrition grinding, etc.), and there is no guarantee that the molecular weight in final products will be within a given range, based on current regulations. Beyond this are the difficulties to obtain stable, homogeneous solutions, even after standard solubilization and purification methods have been used. It still appears to be an open question to what extent the molecular weight of galactomannans depends on the botanical source, as opposed to other possible sources of variation. There is some evidence based on recent SEC measurements that the molecular weights of different galactomannan types are not the same, within typical experimental errors (Brummer, Cui, & Wang, 2003; Daas, Grolle, Vliet, Schols, & de Jongh, 2002; Picout, Ross-Murphy, Errington, & Harding, 2001; Picout, Ross-Murphy, Jumel, & Harding, 2002; Richardson, Willmer, & Foster, 1998), although these studies do not address this question in detail. Since average DG and the associated patterning are considered to be genetically controlled, based on the enzymes catalyzing polymerization, it seems likely that M_w and the associated distribution will also be controlled at a similar level, and then we may speak meaningfully of a species-characteristic value of M_w and molar mass distribution of galactomannans, as well as DG.

Multidetection SEC measurements were originally begun to characterize galactomannans of commercial interest, in particular to determine their molecular weight and to further relate M_w to their intrinsic viscosity $[\eta]$. Even though the empirical $[\eta]$–M (i.e., MHS) relationships have been determined in numerous studies, only multidetection SEC can determine both molecular parameters simultaneously and independently, whether for separated near-monodisperse fractions or the global averages. For nonionic aqueous thickeners such as galactomannans, the intrinsic viscosity takes on particular importance in real applications, as solution viscosity depends primarily on this chain parameter (e.g., $\eta_o \sim ([\eta]c)^{1.4}$ for dilute solutions and $\sim c[\eta]^{3.3 \pm 0.3}$ for $c > c^*$ (Morris, Cutler, Ross-Murphy, & Rees, 1981) and thus only indirectly to molar mass via the coil-expansion rules for a random coil (e.g., $[\eta] \sim M^{0.72}$ for guar galactomannan (Beer, Wood, & Weisz, 1999)). It is well known that galactomannans exhibit some variations in their suspending and thickening properties even for highly pure samples, a fact often attributed to direct result of molar mass and/or chain length differences, yet $[\eta]$ could also result from coil expansion or contraction due to the influence of galactose groups.

In this publication, we describe the results of a comparative SEC study examining galactomannans extracted manually from seeds of two of the three legume subfamilies, Caesalpinioideae and Faboideae. We include galactomannans of commercial interest (these including *C. siliqua* 'carob' or 'LBG' and *Caesalpinia spinosa* 'tara' from the former subfamily, and *C. tetragonoloba* 'guar', and *T. foenum-graecum* 'fenugreek' from the latter) as well as non-traditional sources of galactomannans from seeds that are readily available. We have developed a gentle method for endosperm extraction and milling, and use a standard method for powder milling and further solubilizing, methods which provided the highest possible viscosity and M_w on dissolution according based on extensive optimization (Pollard et al., 2008). Checks on these methods have been repeated here in some instances, and this, in combination with measurements of comparable industrial samples, as well as purified samples, have led us to suggest that the galactomannans 'native' chain structure is preserved to a much better extent by this method. In addition to determination of M_w and $[\eta]$, the molecular weight distributions were determined based on a straightforward data analysis, and SEC fraction data was analyzed to obtain estimates of the unperturbed parameter K_Θ, and thereby the Flory chain stiffness C_∞ and chain persistence length L_p. We have also determined the $[\eta]$–M (i.e., MHS) and R_v–M relationships from SEC fractions as well as ultrasonicated fractions based on the multidetection approach, which will facilitate comparison with literature data.

2. Experimental

2.1. Seeds

Certified guar seeds (Lewis cultivar) were provided by Texas Foundation Seed, Vernon, TX, USA. Tara seeds and pre-processed guar endosperms for bulk measurements were provided by Unipektin AG, Eschenz, Switzerland. Honey locust seeds were collected locally in October 2006 in Oerlikon, Switzerland, and carob (locust bean) seeds were collected in April 2006 from an isolated tree in Mallorca, Spain. Fenugreek seeds were purchased in August 2006 from a local Indian food store. The remaining seeds were purchased from Sandeman Seeds, Lalongue, France. Industrial guar samples were Cargill Viscogum MP41230 (Blattmann Schweiz AG, Wädenswil, CH), Ecopol 1144 (Economy Polymers and Chemical, Houston, TX, USA), Fluka, Sigma G4129, Hercules Supercol U (Hercules, Inc., Wilmington, DE, USA), Provisco Provigel NAG903 (Provisco AG, Hauptwil, CH), Rhodia Meyprodor 400 (Danisco A/S, Copenhagen, DK), TIC VG506893 and G506858 (TIC Gums, Belcamp, MD, USA), Unipektin GH-200, A, and D (Unipektin AG, Eschenz, CH), Wolff 6382–200 (Sugro AG, Basel, CH). Commercial carob sample was Unipektin L-175, commercial tara gum sample was Unipektin SP-175, and commercial fenugreek was Adumim Fenupure (Adumim Food Ingredients, Israel).

2.2. Swelling tests, endosperm extraction, and powder preparation

The necessary conditions to extract endosperms from swollen seeds were determined by monitoring seed mass during soaking at room temperature over 3 days, or during soaking in boiling water over the course of several hours. Because seeds differed with respect to the efficiency and degree of swelling, three methods were used in practice: (1) swelling at room temperature; (2) holding at 95 °C for 5 min followed by room temperature swelling; (3) extended holding at 95 °C and removing swollen seeds at 1 h intervals (Table 1). Seeds having gummy or pasty endosperms or darkened germs after these treatments were discarded.

Powders were prepared by precutting the extracted endosperms to ca. 1 mm size, then hydrating the extracted endosperms in water, and milling the particles with a centrifugal rotor mill (Retsch ZM-200) to pass a 0.2 mm mesh. Powders were then dried under room temperature and humidity conditions for several days and then further dried in a desiccator. Selected galactomannans were further purified using a simple method. The powders were dissolved at ca. 0.5% (w/w) concentrations in deionized water at room temperature, hydrated for 1 h, held in a water bath at 85 °C for 10 min, then centrifuged to remove insoluble matter (40,000*g*, 15 min, 25 °C). The supernatant was precipitated into 2 volumes of ethanol, pressed and rewashed several times with ethanol, dried overnight in a hood with circulating airflow, then further dried in a desiccator. Per cent yields of purified galactomannan were 23–60% (Table 1).

2.3. Multidetection SEC

2.3.1. Instrument and methodology

The SEC instrument is a Viscotek TDA 302, with inline light-scattering (7° (LALS), 90° (RALS)), refractive index (RI), and differential bridge viscometer (DP) detectors. Other equipment includes:

Table 1
Molar mass and intrinsic viscosity analysis of galactomannan polysaccharides derived from *Caesalipiniodeae* and *Faboideae*, determined by multidetection aqueous SEC.

Galactomannan Origin	Legume subfamily	Endosperm extraction method	% SEC recovery	% Yield on purification	Degree of galactose substitution, Reference	$M_w/10^6$ (g/mol)	$[\eta]/10^2$ (ml/g)	M_w/M_n (–)
Caesalpinia pulcherima	Caes.	b	52.9	58.8	0.35, Andrade, Azero, Luciano, and Gonçalves (1999)	1.3	16.4	1.5
Caesalpinia spinosa (tara)	Caes.	b	63.6	–	0.35, Daas et al. (2002)	1.4	17.3	2.0
Cassia alata	Caes.	b	51.3	–	–	0.86	10.7	3.3
Cassia didymobotria	Caes.	b	63.8	–	–	0.89	12.7	1.9
Cassia fistula	Caes.	c	50.7	53.2	0.30, Lal and Gupta (1972)	1.1	16.1	3.1
Cassia grandis	Caes.	c	59.6	58.5	0.32, Joshi and Kapoor (2003)	1.1	15.7	1.6
Cassia javanica	Caes.	c	56.2	59.6	0.31, Andrade et al. (1999)	1.2	17.2	1.3
Cassia laevigata	Caes.	c	51.3	–	–	1.1	14.1	1.9
Cassia multijuga	Caes.	c	52.1	–	0.44, Rechia, Sierakowski, Ganter, and Reicher (1995)	1.2	16.8	1.6
Cassia nodosa	Caes.	c	61.0	–	0.29, Kapoor (1994)	1.3	17.2	1.5
Cassia renigera	Caes.	c	67.0	–	–	1.2	16.9	1.6
Cassia spectabilis	Caes.	b	21.4	–	0.38, Kapoor et al. (1998)	2.1	18.9	1.3
Ceratonia siliqua (carob/ locust bean)	Caes.	b	53.1	–	0.30, Daas et al. (2002)	1.0	15.7	1.6
Gleditsia triacanthos (honey locust)	Caes.	b	42.5	–	0.41, Egorov, Mestechkina, and Shcherbukhin (2003)	1.6	18.2	1.5
Parkinsonia aculeata	Caes.	b	38.4	37.8	0.38, Tewari, Singh, and Gupta (2005)	0.99	13.2	1.7
Parkinsonia africana	Caes.	b	42.8	41.3	–	1.2	16.3	1.2
Cyamopsis tetragonoloba (guar)	Fab.	a	64.8	–	0.65, Daas et al. (2002)	2.6	18.1	2.0
Sesbania egyptica	Fab.	a	27.4	36.1	0.60, Bhattacharyya, Das, Banerji, and Farooqi (1983)	3.1	19.9	3.5
Sesbania grandiflora	Fab.	a	23.2	22.6	0.50, Srivastava, Singh, and Subba Rao (1968)	3.0	19.4	6.2
Sesbania sesban	Fab.	a	44.0	40.2	–	2.4	17.5	1.9
Trigonella foenum-graecum (fenugreek)	Fab.	a	–	–	0.95, Brummer et al. (2003)	3.0	16.1	1.3

[a] Room temperature swelling.
[b] Held at 95 °C for 1 h followed by room temperature swelling.
[c] Held at 95 °C until swollen.

pump VE-1121, degasser VE-7510, autosampler VE-5200, software OmniSEC 4.2 (Viscotek, Houston, TX, USA). A guard column and mixed bed TSK GMPWXL column (Tosoh Bioscience) were used as the stationary phase (hydroxymethacrylate resin). The mobile phase was 0.1 M $NaNO_3$ (aq), made with nanopure quality water (Nanopure Diamond, Banstead, Dubuque, Iowa, USA), prefiltered (Millipore filter type GV, pore-size 0.22 μm). Sample injection volume was 0.1 mL, and samples were run at 0.55 ml/min at 35 °C. Specific refractive index increment (dn/dc) was determined to be 0.155 ml/g for a purified guar galactomannan using the peak-area method without column attached, and 0.158 ml/g with columns attached. The former value was used for all galactomannans, as it closely approximates the median value (0.153 ml/g) reported for guar obtained by more precise methods (0.146 ml/g (0.05 M $NaNO_3$, 633 nm), Funami et al. (2005); 0.1505 ml/g (0.2 M $NaNO_3$, 633 nm), Burke, Park, Srinivasarao, and Khan (2005); 0.155 ml/g (633 nm), Simonet, Garnier, and Doublier (2002); 0.163 ml/g (436 nm), Antonov, Lefebvre, and Doublier (1999); 0.153 ml/g (633 nm), Vijayendran and Bone (1984); 0.135 ml/g, 0.140 ml/g Robinson, Ross-Murphy, and Morris (1982); 0.143 nm, Doublier and Launay (1981). For straightforward data analysis dn/dc has been assumed in this study to be independent of DG as well as chain length.

Detector calibrants were pullulans from Shodex P-82 Standards kit (Showa Denko KK, Japan). Vendor-supplied calibration values were: P-200 (M_w = 212,000, $[\eta]$ = 0.731 dl/g, M_w/M_n = 1.13) and P-400 (M_w = 404,000, $[\eta]$=1.245, M_w/M_n = 1.13), and we used dn/dc = 0.152 (de Noy, Besemer, and van Bekkum, 1996). Standards were prepared from the same stock solution, frozen in plastic vials for long-term storage, and then defrosted before measurement. Over a period of 1 year, fluctuation of calibration constants based on this method were 1.4%, 4.4%, 4.4%, and 1.8% relative standard deviation for RI, LALS, RALS, and DP detection, respectively.

Galactomannan solutions were prepared by hydrating the crude powders in the eluent solvent for 1–2 h at 0.20–35 mg/g (to achieve ca. 0.15 mg/g polysaccharide concentration), submerging the vials in a water bath for 30 min at 85 °C, then cooling and filtering through 0.46 μm PTFE filters before injection. The galactomannan concentration after chromatographic separation was obtained from the RI signal assuming dn/dc = 0.155 ml/g, in preference to a user-supplied concentration; this eliminates one factor dependence of M_w on dn/dc and improves precision (Reed, 1995). Per cent recovery of the sample through the columns was 16–67%, based on assumed dn/dc, RI peak area, and crude concentrations (Table 1). The per cent recovery values reflect mainly three factors, in order of importance: (1) the galactomannan content and purity of the original powders, (2) insufficient solubilization, and (3) loss of sample within the column due to adsorption, etc. The per cent recovery values are in excellent agreement with the galactomannan yield on purification (Table 1), although this check was limited to about half the samples investigated; galactomannan content of the crude powders is thus the main reason for per cent recovery values less than 100%. Statement 2 is justified based on the finding that losses due to insufficient solubilization can be up to ca. 15% based on our standard method, but molar mass fractionation is not important (see below); solubilization losses are thus a minor but systematic effect. Statement 3 is based on the recovery values of ca. 100% of purified galactomannans (see below). Additionally, we attempted to quantify whether sample is lost at the LALS filter. Based on peak areas of a pullulan standard and a purified guar, the method detected ~2% loss of sample, presumably occurring by deposition of chains at this filter. It was not possible to verify col-

umn losses per se, or the characteristics of individual samples, but we believe the mass loss is small and must be accepted as a limitation of the aqueous SEC method.

For purified samples, the solubilization method was identical except the room temperature soak time was extended to 1 day, and samples were then held at 85 °C for 15 min before filtration; here per cent recoveries from the column were 87–104%, in good agreement with the expected purity of these samples.

2.3.2. M_w, $[\eta]_w$, R_v, and molar mass distribution

Weight-average molar mass M_w and intrinsic viscosity $[\eta]_w$ were determined using OmniSEC 4.2 software. Only the 7° detector was used for M_w calculation (LALS), since otherwise an extrapolation to 0° takes place using only two detector angles. For large molecular sizes, M_w determination by LALS is still subject to bias from intramolecular scattering. The relevance of this bias for the galactomannans was estimated using the Debye structure factor for a random coil, $P(q)$, and the working equation for inline M_w determination, $Kc/R_\theta = (M_w \cdot P(q))^{-1}$. Taking $R_g = 146$ nm, as reported for guar galactomannan by static light-scattering in aqueous solution at a similar temperature (Simonet, Garnier, and Doublier, 2002), this gives $P(q) = 0.99$ ($\lambda = 670$ nm, $\theta = 7°$) or about 1% estimated bias; 3% bias is estimated based on the largest molecular sizes in the chromatograms (est. $R_g \sim 250$ nm).

It was found with offline checks that this software incorporates LALS signal smoothing, as well as an interdetector volume correction and deconvolution of differential pressure signal in order to minimize broadening artifacts; the latter two manipulations are out of user control and not described in detail as far as we are aware. However, all further data analyses were accomplished offline, and checks of M and $[\eta]$ were in agreement with that obtained with the working calibration equations. Local viscometric radius R_v was calculated using the Einstein relation to represent chain size by a hydrodynamic-equivalent sphere:

$$R_v = 10^7 \left[\left(\frac{3}{10\pi} \right) [\eta] \frac{M}{N_A} \right]^{\frac{1}{3}} \quad (1)$$

where M is the local molar mass [g/mol], $[\eta]$ is the local intrinsic viscosity [ml/g], N_A is Avogadro's number [mol^{-1}], and R_v is the equivalent viscometric radius [nm].

The 'SEC' distribution $w(\log M)$ was calculated based on the following formula:

$$w(\log M) = -\frac{1}{d(\log M)/dV_r} \frac{S(V_r)}{\int S(V_r) dV_r} \quad (2)$$

where $S(V_r)$ is the baseline-subtracted refractive index signal, $\log(M)$ is a 1st order polynomial function fitted to $\log(M) = f(V_r)$, and $d(\log M)/dV_r$ is the slope of this function with respect to retention volume V_r (Shortt, 1993). To determine the molar mass distribution in a transparent manner, $d(\log M)/dV_r$ was obtained by a linear regression of $\log(M)$ over a defined portion of the peak center, ignoring curvature at peak edges, and then extrapolated to cover the full chromatograms (here $V_r = 5.5$–10.0 ml). This treatment is illustrated in Fig. 4 for two *Cassia* galactomannans and two *Sesbania* galactomannans. The weight-fraction distribution of molar mass $w(M)$ was then obtained by:

$$w(M) = \frac{1}{\ln(10)} \frac{1}{M} w(\log M) \quad (3)$$

For comparison to theoretical models, the Schulz–Zimm (SZ) model was used (Peebles, 1971; Schulz, 1939; Zimm, 1948). The SZ differential weight-fraction is:

$$w(x) = \frac{1}{\Gamma(k+1)} (-\ln p)^{k+1} x^k p^x \quad (4)$$

where x is the chain length, p is the reaction probability (chain growth polymerization) or extent of reaction (condensation polymerization), Γ is the gamma function, and k is the chain coupling parameter reflecting the mode of chain termination. If $k = 1$, the SZ distribution reduces to the Flory 'most-probable' distribution (Flory, 1936), and then has only one scale parameter for high M. To compare with experimental data, a parameterization based on the peak of the distribution M_p was used, as this can be obtained directly from the data as a fixed parameter:

$$w(M) = \frac{1}{\Gamma(k+1)} \left(\frac{k}{M_p} \right)^{k+1} M^k \exp\left(-\frac{k}{M_p} M \right) \quad (5)$$

Fits were obtained between M_p and the high-M cutoff value to a minimum in chi-square, using fixed M_p, and floating k.

2.3.3. $[\eta]$–M and R_v–M relationships

The relationships $[\eta] = K_l \eta_l M^a$ and $R_v = K_{Rv} M^v$ were determined from SEC fraction data (M, $[\eta]$) and from ultrasonicated fractions (M_w, $[\eta]_w$) using offline analysis. A fixed region centered on the RI signal peak, where S/N is high, was employed to determine the exponent and prefactors of these relationships reproducibly. For native galactomannans, the relationships are valid for narrow molecular weight ranges, about 1.5–3 million g/mol for the Faboideae galactomannans and about 0.8–1.6 million g/mol for Caesalpinioideae. For ultrasonicated galactomannans, the relationships cover about a decade in molecular weight, from 100,000 g/mol up to the native M_w. To incorporate DG-dependent prefactors, we have used $[\eta]_{Galactomannan} = [\eta]_{Mannan} \times M_{backbone}/M_o$, where $M_{backbone}$ is a constant for galactomannans, 162 g/mol, and M_o is the repeat unit mean molar mass ($\approx 162 \times DG + 162$).

2.3.4. Chain flexibility and persistence length

Intrinsic chain flexibility was determined based on estimates of the unperturbed parameter K_Θ, defined under theta-conditions by

$$[\eta]_\Theta = K_\Theta M^{0.5}, \quad K_\Theta = \phi_0 \left(\frac{\langle r^2 \rangle_0}{M} \right)^{\frac{3}{2}} \quad (6)$$

where ϕ_o is Flory's universal constant for random-coil polymers (2.6×10^{26} mol^{-1}) and ($<r^2>_o/M$) is the unperturbed dimension. The unperturbed parameter was estimated from good solvent data using the Burchard–Stockmayer–Fixman (BSF) plot. By this method, if plots of $[\eta]/M^{0.5}$ vs. $M^{0.5}$ are linear for a linear, high-M random-coil polymer, then the intercept gives directly K_Θ. M and $[\eta]$ were obtained from SEC fractions. The intrinsic stiffness of the galactomannan glycosidic bond is then obtained through the dimensionless Flory ratio C_∞, defined by:

$$C_\infty \equiv \frac{\langle r^2 \rangle_0}{nl^2} = \frac{\langle r^2 \rangle_0}{(\frac{M}{M_0}) l^2} = \left(\frac{K_\Theta}{\phi_0} \right)^{\frac{2}{3}} \frac{M_0}{l^2} \quad (7)$$

where the numerator refers to unperturbed chain dimensions and the denominator refers to chain dimensions of a random-flight chain, taking $l = 0.54$ nm. The substitution $M = M_o n$ has been made, where M_o is the repeat unit mean molar mass and n is the degree of polymerization. Persistence length was calculated based on the wormlike chain approximation: $L_p = C_\infty \times l/2$.

3. Results

3.1. Manual endosperm extraction by swelling

Endosperms were manually separated from seeds by swelling them in excess tap water for the minimum time and lowest temperature necessary to soften the seed and allow manual removal of the germ and seed coat, as described previously (Pollard et al.,

2008). It should be noted other manual extraction methods have been used to isolate galactomannans from seeds, for instance involving acid-treatment to remove the outer seed coat layer, or often milling of the entire seed. We have avoided these methods for the principle reason that we are interested to isolate only those galactomannans within the endosperm itself, and to do so with minimal extraneous treatment. The acid-treatment method may or may not fully remove the seed coat layer and in fact the cell layers adjoining the testa are not removed in a reproducible manner with this method. Milling of entire seeds is even less suitable, as this requires subsequent enzyme deactivation, following by the necessary sifting methods to eliminate all remaining germ and seed coat; this is clearly not a trivial exercise to accomplish on the lab scale.

On extended soaking or boiling, all the *Cassia* seeds exuded a gel-like substance from the seed coat, and further seed swelling could only take place after this layer was removed. Because of the variable rates at which this took place, a wide range of swelling rates and temperatures were required for these seeds in contrast to the others. Carob seeds notably did not display this characteristic and absorb water proceeding initially from the hilum end; the best way to swell these seeds is to apply a shock treatment, holding for 5 min at 95 °C water bath, following by room temperature swelling (Pollard et al., 2008). On the other hand, all Faboideae seeds required only room-temperature swelling, and these seeds appear to swell uniformly.

The seeds of *C. alata*, *C. didymobotria*, and *C. laevigata* had divergent swelling behavior and seed characteristics. The first of these had soft endosperms, in direct contrast to all other *Cassia* seeds; the second of these had brown germs; the third of these contained both brown and green seeds, the former containing significantly contracted endosperms, more rapid swelling rates, and much smaller seed sizes than green counterparts. The latter two samples also had bimodal molecular weight distributions, and we speculate that these seed lots had begun germination and subsequently dried out before purchase. We have therefore rejected these galactomannans from further consideration, as they are probably unrepresentative of the native structure.

3.2. Optimum solubilization method

The optimum method for solubilizing galactomannan from crude endosperm powders (as well as milling protocols) were determined by extensive optimization using rheometry and SEC data in a previous study (Pollard et al., 2008). The method involved a defined time–temperature treatment by heating crude LBG solutions at 85 °C for 30 min, which dissolves most of the polysaccharide, minimizes solvent-fractionation effects (characteristic of the low-DG galactomannans when dissolving at room temperature) and limits degradation. The method has been adopted here for the other galactomannans powders as it is simple to implement as a standard protocol. We have further checked here whether the 30 min soaking time at 85 °C is sufficient to dissolve the galactomannan fully or cause backbone hydrolysis. Three of the milled powders *C. fistula*, *C. tetragonoloba*, and *T. foenum-graecum* were held at 85 °C for extended time periods, and concentration and molecular weight were monitored by SEC, as indicators of dissolving kinetics and degradation.

In Fig. 1, the peak areas obtained from RI traces as a function of hold time are illustrated for these samples, and in Fig. 2, the corresponding data for M_w and $[\eta]$ are shown. In each figure, the data are supplemented with raw RI traces and the molar mass distribution obtained for guar, as an example how the data were obtained. It is clear on examination of both figures that our solubilization method makes a tradeoff to enhance dissolution at the expense of slight hydrolysis. Although the data here might be further modeled, we accept the 30 min hold time as a good tradeoff, as found in the previous study. In the example of guar, M_w is depressed by ≈5% by backbone hydrolysis while achieving 87% of the final concentration. It appears that *C. fistula* is somewhat more resistant to hydrolysis than the other two samples. Significantly, molar mass fractionation does not seem to occur to a major extent. This is clear from examining both the RI traces and distributions, which do not shift to a great extent before the effects of hydrolysis are registered. Therefore, minor solubilization losses, as such, may be tolerated to some extent as a limitation of the treatment.

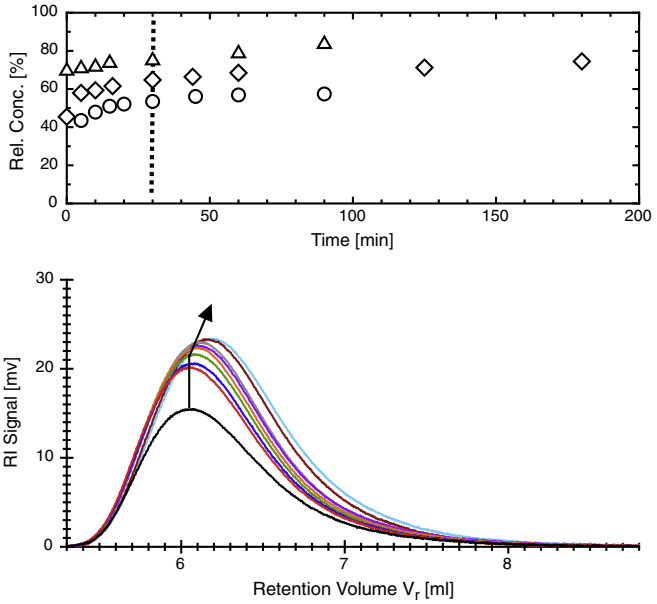

Fig. 1. Influence of solubilizing treatment at 85 °C on galactomannan concentration of *C. fistula* (circles), *C. tetragonoloba* (diamonds), and *T. foenum-graecum* (triangles). Lower plot illustrates raw RI traces for *C. tetragonoloba*.

Other solubilization regimes may be considered and have been developed (e.g., the pressure-bomb technique, Picout et al., 2001), but we feel we have chosen a standard method that balances several competing effects that are otherwise difficult to control, and can be implemented in a straightforward manner without special equipment. As a simple statement of uncertainty regarding the solubilization treatment, we expect no more than about 5% depression in M_w and $[\eta]$ reported in this study based on the solubilizing treatment, neglecting other biases.

3.3. Repeatability and reproducibility of aqueous SEC

The reproducibility of aqueous SEC is often poorer than found in organic solvent SEC, but we have found that most problems reside with system stability (i.e., the instrument is not optimized for aqueous systems), not sample measurement per se. The most significant measurement problems we have found relate to effects from very large molecular sizes, such as guar ($R_g \approx 146$ nm, Simon-et et al., 2002), where it is important to carefully optimize operating parameters.

We assessed reproducibility of M_w and $[\eta]$ by checking historical data obtained for identical samples of guar over a one year period, over which time calibration constants fluctuates and the column deteriorates. Here, we have found for guar galactomannan $M_w = 2.5 \pm 0.1$ million g/mol, and $[\eta] = 18.2 \pm 0.7$ dl/g, or about 4% relative dispersion, for five separate measurements where the standard solubilization method was applied. This dispersion is of a similar magnitude as that obtained from the calibration itself. The reproducibility of scaling exponents for this sample was found

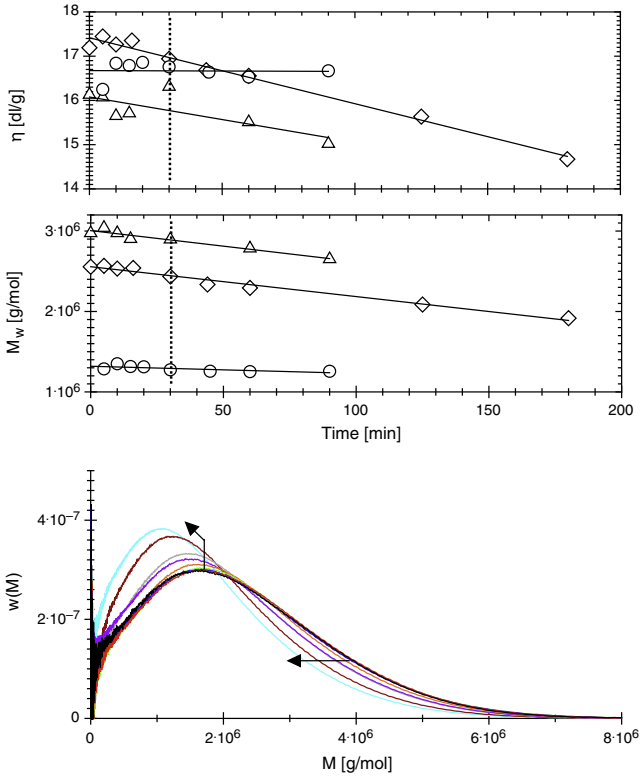

Fig. 2. Influence of solubilizing treatment at 85 °C on galactomannan M_w and $[\eta]$. Symbols as in Fig. 1. Lower plot illustrates molar mass distributions for *C. tetragonoloba*. A 30 min hold time was chosen as a compromise to promote rapid dissolution while limiting thermal hydrolysis.

to be $[\eta] \sim M^{0.60\pm0.01}$ and $R_v \sim M^{0.53\pm0.01}$. For molecular weight distributions, we show in Fig. 3 seven datasets obtained for this sample, two of which were rejected because of clearly shifted distributions and lower M_w (down to 1.8 million g/mol in one case). We subsequently confirmed that shear degradation occurring within the guard column was the most likely cause, by comparison to dextran and pullulan standards with a fresh guard column. Notably, guard column deterioration was not evident from the usual checks with primary calibrants. In a second case, short-term reduction of M_w had no identifiable cause. This is clearly a problem for long-term reproducibility of the separation method for very large random-coil polysaccharides such as guar; the molecules seem to be a very sensitive to the separation process, even when operating at minimum stable flow rate and lowest manageable solution concentrations to avoid viscometric artifacts (here 0.55 ml/min, and $c \sim 0.15$ mg/ml). For this reason, we have also used guar galactomannan as secondary calibrant for checking system stability, and require that M_w be maintained within 10% of 2.5×10^6 g/mol before accepting the measurement as valid.

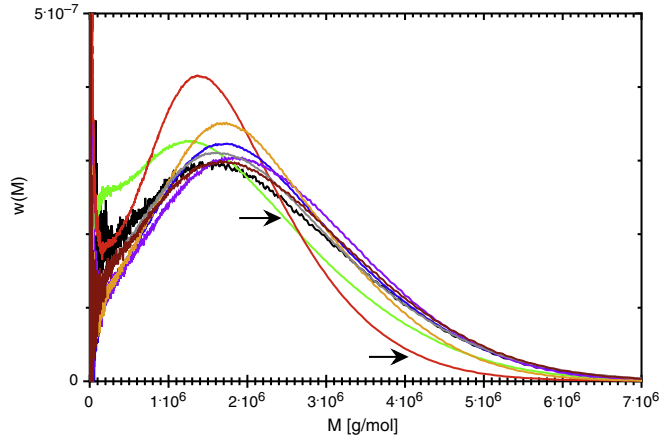

Fig. 3. Reproducibility of molar mass distributions of guar galactomannan over a period of 1 year. Arrows mark two samples showing evidence of shear degradation.

We have also assessed repeatability of M_w and $[\eta]$ values through repetitive measurements over a short time period using otherwise identical operating procedures. For six injections of the same solution of guar, the relative standard deviation was 1.9%, 0.9%, and 3.9% for M_w, $[\eta]$, and RI peak area, respectively. Errors of this magnitude indicate a high repeatability when the chromatographic system is stable, and reflect typical values found for a measurement series during a short time period (e.g., data in Table 1).

3.4. Chromatographic separation of native galactomannans

In Fig. 4 the refractive index traces of native unpurified galactomannans are presented along with the universal calibration curve. These elution traces indicate that all the native galactomannans had unimodal distributions, except *C alata* and *C didymobotria*. We believe these seeds were damaged before purchase, and have therefore rejected them as unrepresentative. Further work may needed to justify this statement even though, to our knowledge, it is not trivial to ascertain the history of seed lots prior to purchase unless they are available locally.

Clustering of the RI traces according to the botanical origin of the galactomannans is clearly visible. The Faboideae galactomannans elute at lower retention volume V_r, and have correspondingly higher viscometric radii than the *Cassia*-derived galactomannans. It is shown later that this results from the dramatically higher M_w as well as slightly higher $[\eta]_w$ of these galactomannans than their Caesalpinioideae counterparts.

As a check on whether galactomannans are separated by size, we have determined an apparent universal calibration curve from ultrasonicated galactomannans of varying chain composition, obtaining hydrodynamic volume and viscometric radius from in-line detection (via M_{peak} and $[\eta]_{peak}$). This alternative approach was necessary because aqueous SEC standards have small molecular sizes in aqueous solution despite their high molar mass (e.g., dextran: $M_w = 2.2 \times 10^6$ g/mol, $R_v \approx 25$ nm; pullulan P-800: $M_w = 8 \times 10^5$ g/mol, $R_v \approx 30$ nm), and thus the calibration range is insufficient; the native galactomannan polysaccharides have considerably larger molecular sizes (e.g., guar: $M_w = 2.5 \times 10^6$ g/mol, $R_v = 86$ nm). According to the principle of universal calibration, each fraction should elute at retention volume V_r according to $\log(M[\eta])$ or $\log(R_v)$, for chains of differing composition. As shown in Fig. 4, this principle is followed for molecular sizes $R_v = 15$–100 nm, indicating an approximately log-linear relationship characteristic of broad pore-size columns.

The native samples elute at a V_r in good agreement with the universal calibration curve, although the pullulan standards elute slightly earlier than expected. This has been found to be reproducible, and therefore could indicate either a minor systematic error in the LALS measurement of galactomannans or, more likely, the presence of weak, secondary interactions within the column, possibly the influence of salts or oligomeric sugars within the native samples. Nevertheless, the high linearity of the calibration curve itself indicates that any secondary interactions are not preferential towards the different galactomannans' chain structure, as here DG varies from 0.29 up to 0.95. Therefore, we can conclude that the native galactomannans elute principally according to a size-exclusion mechanism.

A similar approach can be used to check whether individual fractions of a given sample elute according to universal calibration

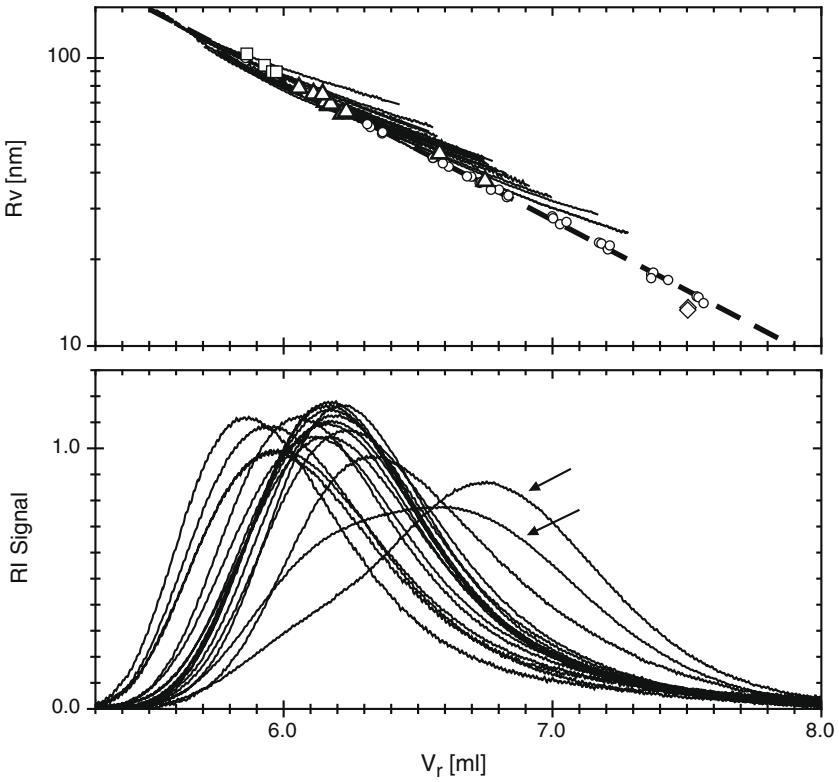

Fig. 4. Chromatographic separation of unpurified galactomannans derived from legume seed endosperms (TSK mixed bed columns, 35 °C, 0.1 M $NaNO_3$(aq)). Upper graph: Faboideae galactomannans (squares), Caesalpinioideae galactomannans (triangles), pullulan standard (diamonds), universal calibration with fit (circles + dashed line), and local viscometric radius R_v from inline detection (lines). Lower graph: normalized refractive index traces. Bimodal distributions of *C. alata* and *C. didymobotria* are marked with an arrow.

principles, bearing in mind that local signals becomes less reliable near peak edges. 'Local' values of R_v obtained from inline molar mass and intrinsic viscosity detection are also shown as lines in Fig. 4 for the native galactomannans after separation. Here, these effective calibration curves have less steep slopes than obtained from the universal calibration. We have attributed this to band broadening, as the sample's chromatographic peak widths are comparable to those of the narrow pullulan standard. Outside of the peak region, moderate deviations from the expected log-linear dependence are also observed in the local signals. Weak upturns on the leading edge of the peaks (V_r < 6 ml) indicates possibly pore exclusion of the largest molecules. The manufacturer claims that the column's resolution limit is 8 million Da for polyethylene oxide, and thus $R_v \approx 140$ nm based on published MHS constants (Armstrong, Wenby, Meiselman, & Fisher, 2004). This agrees with the slight upturn observed for the large components within the Faboideae samples, and in this case it is a small proportion of the total chain mass. Unfortunately the lack of larger pore-size columns or appropriate size-based standards make this a difficult statement to verify. On the trailing edge of the peak, upturns also occur to a slightly differing extent depending on the sample. Many other studies have reported deviations from log-linear behavior for several different polysaccharide types (see for example, Vold, Kristiansen & Christensen, 2006). Explanations for this deviation have included: nonexclusion behavior due to secondary interactions within the column, the late elution of aggregated chains or compact insoluble particles that may bias the detector signals, pollution of the mobile phase by the slow release of large chains trapped at filters/frits, or simply low S/N. In the study by Vold et al. (2006), ultracentrifugation was successful to partially eliminate the upturns. However, we found that purified galactomannans exhibited the same deviations as the unpurified samples, and we were further not able to remove them by centrifugation.

As an illustration of the raw data signal obtained from multidetection SEC, four representative galactomannan chromatograms are shown in Fig. 5, two from Caesalpinioideae subfamily and two from Faboideae. To derive the molar mass distribution, the dotted lines are shown where extrapolation is required to cover the full chromatogram, where otherwise light-scattering detection is insensitive.

3.5. M_w, [η], and molar mass distribution

Based on the standard data analysis, the Faboideae galactomannans were found to have two to three times higher M_w than those from the Caesalpinioideae ($M_{w,Fab}$ = 2.4–3.1 × 10^6 g/mol, $M_{w,Caes.}$ = 0.86–2.1 × 10^6), based on the defined extraction and solubilization method (Table 1), differences far exceeding the stated 'reproducibility' error of ca. 4%. In Fig. 6, the 'SEC' distributions $w(\log M)$ are shown, with accompanying plots of the intrinsic viscosity and viscometric radius, and in Fig. 7 the weight-fraction distributions $w(M)$. Since the Faboideae galactomannan have broader absolute peak widths and higher peak maxima than Caesalpinioideae, a categorization is suggested according to the botanical subfamily.

Regardless of the galactomannan type, the distributions appear to have a universal shape: $w(M)_{max}$ decreases in proportion as M_p (or M_w) increases. This indicates that one scale parameter might account for all $w(M)$ variation. Thus it may be more relevant to consider botanical variations in M_w as a result of simple scaling of the distribution, with a more or less constant peak shape. To consider this point, we have compared the data to the one-parameter Flory 'most-probable' distribution, and the

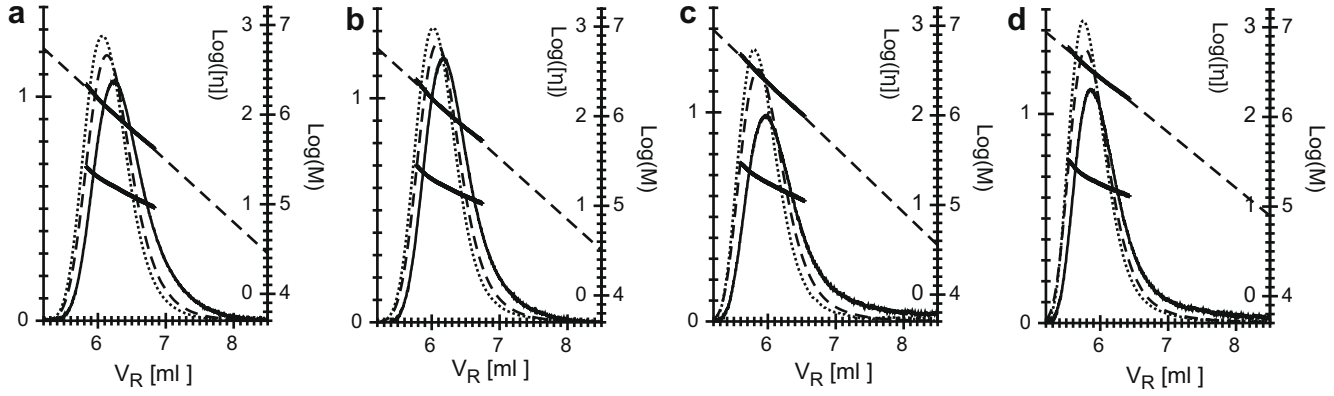

Fig. 5. Illustration of data treatment applied to galactomannans from (a) *C. grandis*, (b) *C. multijuga*, (c) *S. grandiflora*, and (d) *S. egyptica*. Traces are normalized refractive index (line), low-angle light scattering (dot), and differential pressure (dash). Molar mass and intrinsic viscosity from inline detection are illustrated. The molar mass distribution was derived using a fit to log(M) over a defined range of the peak center and extrapolated to cover the full chromatogram range, 5.5–10.0 ml (dashed line).

two-parameter Schulz–Zimm (SZ) distribution, which reduces to the former when the coupling parameter k is 1. Some representative $w(M)$ data are illustrated in Fig. 8, covering the narrowest and widest distributions observed. The Flory distribution ($p \sim 0.9998$) does indeed give peak shapes and scaling behavior in agreement with the datasets though it appears the distribution is too broad to accurately represent the data. A better description was obtained with the SZ model, which allows peak width variation through the parameter k. Reasonable fits to this model were obtained, giving k in the range of 1.5–3. Thus, by the data treatment employed here the distributions are well approximated by the SZ distributions, with apparent distributions narrower than the Flory distribution. The polydispersity values (M_w/M_n), were also less than two for the majority of the samples, but as SEC M_n values are not absolute determinations (and notoriously sensitive to extrapolation methods), some of the higher values are erroneous.

It is interesting to consider the M_w and distributions in light of the galactomannans' respective DG, as they appear to be correlated. As an example, consider four galactomannans that have been widely studied: *C. siliqua* (locust bean gum), *C. spinosa* (tara), *C. tetragonoloba* (guar), and *T. foenum-graecum* (fenugreek). The characteristic average DG of these galactomannans is 0.292, 0.345, 0.654 and 0.95 (Brummer et al., 2003; Daas et al., 2002). The obtained M_w values increase in apparent correlation, 1.0, 1.4, 2.5, and 3.1 million g/mol.

To explore further the possibility that M_w and DG are correlated, we have plotted these molecular parameters against each other in Fig. 9, the latter parameters obtained from literature data where possible. (For one sample, DG is not reported (*S. sesban*) and for three others, there was poor agreement of the reported DG value and that deduced using our MHS model (see below). In these cases the deduced DG values were used (dark symbols).) The correlation between M_w and DG is convincing only for the low-DG Caesalpinioideae galactomannans (DG = 0.29–0.44). A best-fit line has been drawn through the data points indicating a possible relationship between the two molecular parameters. Therefore, galactomannans in general do not represent a homologous series, in the strict sense; however, it does seem possible to summarize with some certainty that Caesalpinioideae galactomannans have linked DG and M_w (and hence chain length). *C. spectabilis* seems to be the only notable exception, with an uncharacteristically high M_w for the Caesalpinioideae galactomannans.

Sesbania aegyptica and *S. grandiflora* had the highest M_w found in this study. These samples originate within the same botanical subfamily and have nearly the same DG as guar, and therefore might be further exploited as mass-efficient thickeners with similar interaction properties. For these and the other galactomannans with very high M_w, multidetection SEC also provides some important size information. The galactomannan molecules with M = 7–8 million g/mol (e.g., the high-M tail of *S. egyptica* and *C. tetragonoloba*) have viscometric radii in water of ca. 180 nm, and thus radii of gyration will be about 270 nm, considering the linear chain architecture. For uncharged random-coil polymers, such molecular sizes are very large indeed and are probably a significant factor in the tendency towards shear-degradation, and difficulty to obtain stable, reproducible SEC measurements.

As a check on the quality of our methods, we have compared the endosperm powders to a corresponding commercially available variety. For the commercial tara and carob samples, M_w and $[\eta]_w$ were around 10% lower, and significantly lower for the fenugreek sample, around 70%. The distributions for these industrial samples are clearly broadened in relation to the manually extracted samples (Fig. 10). In fact, the fenugreek sample appears to have been deliberately hydrolyzed during industrial production. For guar, the observed range in M_w for over 20 products was 1.99–2.56 $\times 10^6$ g/mol, after solubilization for 24 h at room temperature to minimize degradation artifacts; the highest M_w was found for the manually extracted endosperms. These variations might be considered typical for commercial products, and result from native molecules' large size and tendency to degrade under manufacturing conditions. We are not aware of a study that has quantified the magnitude of such effects, and therefore researchers in the field should keep in mind the magnitude of these variations when using such powders as a starting material.

To consider our data in light of those in the literature, we have tabulated the relevant studies in Table 2 (covering only light-scattering and SEC), since recent reviews have not covered M_w values (Gidley & Reid, 1995; Shcherbukhin & Anulov, 1999; Srivastava & Kapoor, 2005). The trend emerging from the studies of Daas et al. (2002), Brummer et al. (2003), and Picout et al. (2002) seems to be that guar has a higher M_w than carob, with which our data also agree. Numerous studies have determined the M_w of guar, however, and here it may be relevant that our determination of M_w is one of the highest values reported (2.5 $\times 10^6$). This may be related to our manual extraction method and particular care to avoid degradation, but also systematic differences in methods, choice of dn/dc, etc. also play a role. Assuming this is relevant for many of the previous studies, we believe our data agrees quite well with the early SEC and light-scattering study by Vijayendran and Bone (1984) who determined M_w by light-scattering (2.1 $\times 10^6$) and SEC (2.2 $\times 10^6$), as well as more recent determinations by Simonet

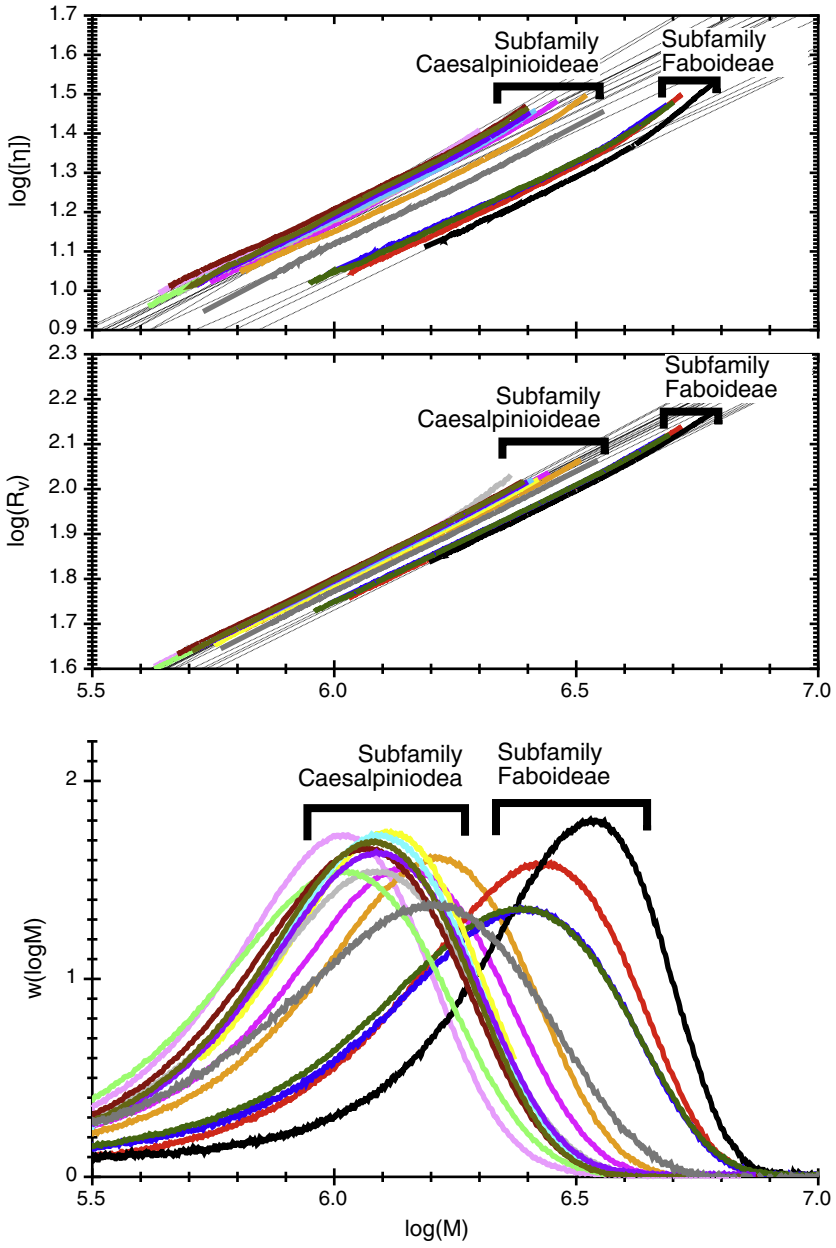

Fig. 6. Scaling of intrinsic viscosity $[\eta]$ (dl/g) and viscometric radius R_v (nm) obtained from inline detection, over a defined region bracketing the chromatographic peak. Lower plot shows 'SEC' distribution w(log M). For all galactomannans investigated, $[\eta] \sim M^{0.61\pm0.02}$, $R_v \sim M^{0.54\pm0.01}$, but strong differences in prefactors are observed depending on degree of galactose substitution.

et al. (2002) by light-scattering (2.17×10^6) and Laguna, Tarazona, and Saiz (2003) by SEC (2.2×10^6).

3.6. $[\eta]$–M and R_v–M relationships

In Fig. 6, the $[\eta] = K_{[\eta]}M^a$ and $R_v = K_{Rv}M^v$ relationships are shown for galactomannans of both botanical groupings. It was possible to determine these relationships over a limited molar mass range where the data are reliable, as illustrated by the accompanying 'SEC' distribution w(log M). The exponents were, within error, the same for all galactomannans ($[\eta] \sim M^{0.61\pm0.02}$ and $R_v \sim M^{0.54\pm0.01}$), but due to variation in the prefactors the lines do not possess a common intercept. Although differences here might be considered on the level of botanical subfamily, on examination it is clear that the curves are shifted both vertically and horizontally in a strict dependence on DG.

We have therefore developed a relationship based on the form suggested in a previous study (Fernandes, Gonçalves, & Doublier, 1991), in which the $K_{[\eta]}$ and K_{Rv} coefficients vary with DG, and exponents are assumed independent of DG. For the SEC fractions, the relationship was found to be approximated by the following model:

$$R_{v,GM} = 0.63 \pm 0.05 \cdot M_0^{-0.54} \cdot M^{0.54\pm0.01}; \quad \text{native } M$$

$$[\eta]_{GM} = 1800 \pm 200 \cdot M_0^{-1.61} \cdot M^{0.61\pm0.02}; \quad \text{native } M$$

where R_v has units nm, $[\eta]$ has units ml/g, and the galactose dependence has been incorporated using the mean molar mass of the repeat unit M_o. The shifting of curves in log–log plots can be identified here as ($M_{backbone}/M_o$) which biases the ordinate $[\eta]$, and a factor M_o^{-a} that biases the abscissa M.

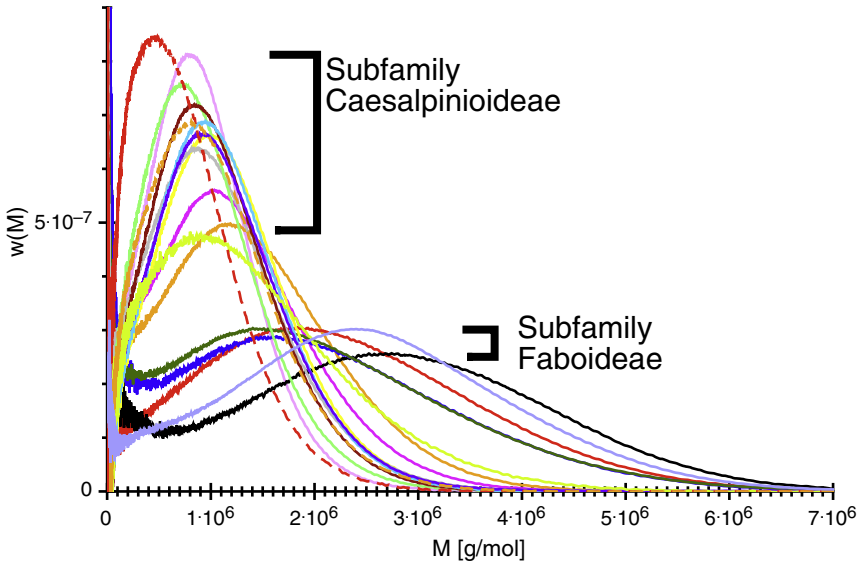

Fig. 7. Weight-fraction distribution of molar mass $w(M)$ of galactomannans from the indicated legume subfamily.

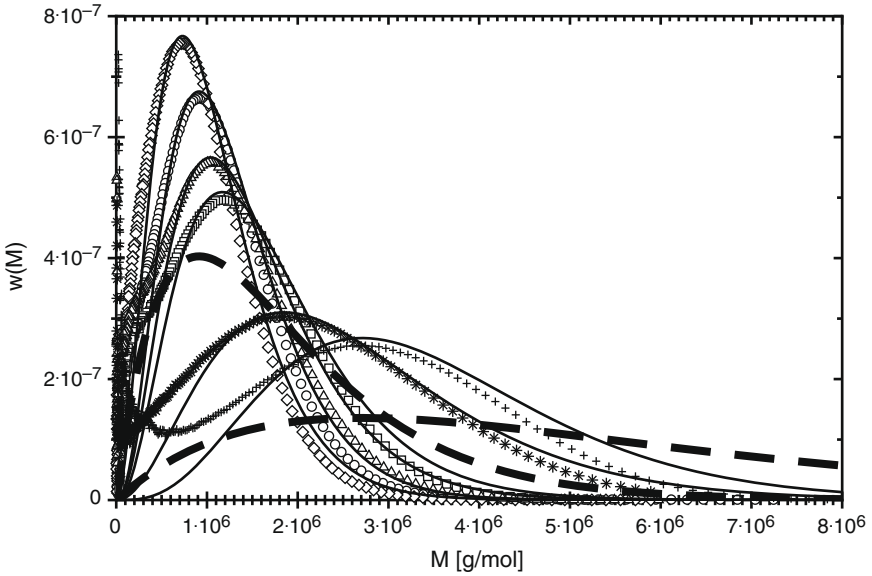

Fig. 8. Fitting of molar mass distributions with Schulz–Zimm model (lines), illustrated for *C. grandis* (diamonds), *C. nodosa* (circles), *C. spinosa* 'tara' (triangles), *G. triacanthos* 'honey locust' (triangles), *C. tetragonoloba* 'guar' (asterisks), and *S. egyptica* (plusses). The Flory 'most-probable' distribution is plotted covering a similar range of peak molar mass (dashed lines).

This model only describes $[\eta]$ and R_v over a narrow molar mass range available from the SEC fraction data. We have also repeated the analysis using the more conventional method, in which M_w is related to $[\eta]_w$ from separate molar mass fractions obtained by ultrasonication. These data are illustrated in Fig. 11 for six galactomannans, each series covering a wide molar mass range. The same features are observed here: nearly identical scaling exponents and strong prefactor differences depending on the galactose content. For the ultrasonicated fractions, we obtained the following:

$$R_{v,GM} = 0.49 \pm 0.05 \cdot M_0^{-0.57} \cdot M_w^{0.57\pm0.01}; \quad M_w \approx 1 \times 10^5 - \text{native}$$

$$[\eta]_{GM} = 730 \pm 100 \cdot M_0^{-1.71} \cdot M_w^{0.71\pm0.02}; \quad M_w \approx 1 \times 10^5 - \text{native}$$

As shown in Fig. 11, the model offers an excellent approximation of the individual fits, and in particular the prefactors are properly accounted for, at least over the given range of DG (0.29–0.95). However, it is also clear that the ultrasonicated fractions have lower prefactors and higher exponents, than obtained directly from the near-monodisperse SEC fractions. This appears to be a result of slight curvature in the log–log plots when determined over a wide M range, a common though not universal finding for many polysaccharides.

To consider the significance of the prefactor shifts, consider that $[\eta]$ is an intensive quantity: for a given high-M chain $[\eta]$ is proportional to the volume pervaded by an individual coil *per unit chain mass*. For a high molecular weight galactomannan with fixed chain length, $[\eta]$ ought to vary in inverse proportion to the total mass of pendant galactose groups; for increasing DG, $[\eta]$ must correspondingly decrease, by as much as a factor of two based on the classical structure of galactomannans. Although it is commonly stated in one form or another that $[\eta]$ depends on the length of the mannose backbone and not on the concentration of side chains, this is erroneous. The incorporation of galactose substituents must force a reduction in $[\eta]$, based on its definition; even though this might

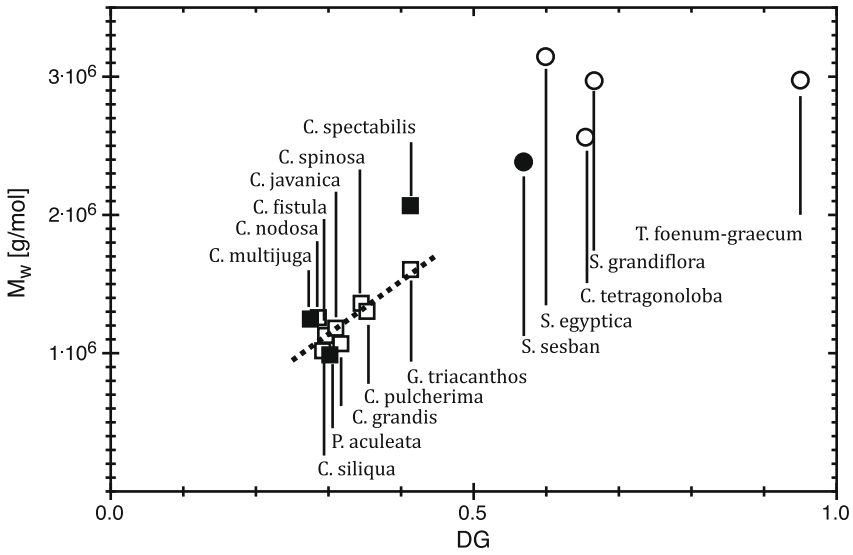

Fig. 9. Schematic plot showing relationships between the degree of galactose substitution DG and weight-average molar mass M_w for galactomannans: Caesalpinioideae (squares), Faboideae (circles). Darkened symbols indicate the use of deduced DG based on the MHS model (see text).

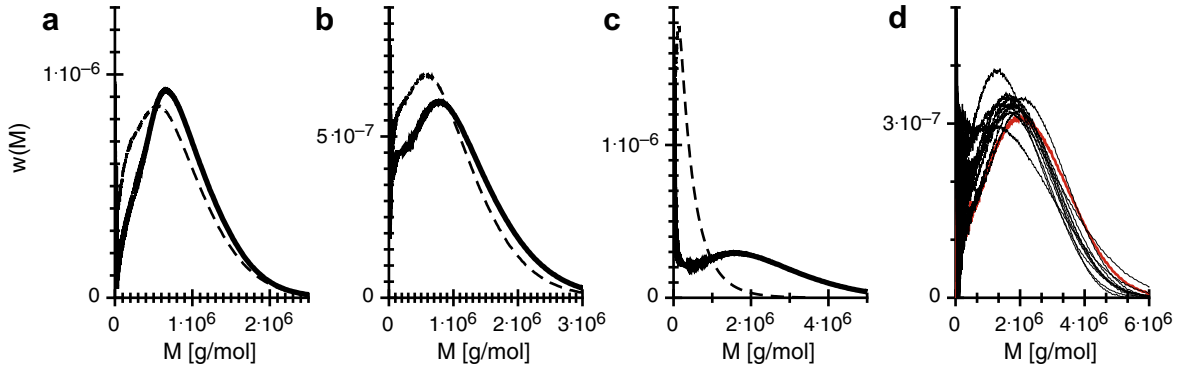

Fig. 10. Comparison of molar mass distributions of industrially-manufactured galactomannans (dashed line) to our manually processed samples (solid line): $w(M)$ for (a) carob, (b) tara, (c) fenugreek, and (d) guar.

be considered an artificial dependence, it is required for an accurate description of the data. Normalized quantities, however, have also been used. For instance McCleary, Amado, Waibel, and Neukom (1981) used $[\eta]m_{gal}$, versus backbone molar mass to compare specific viscosities of galactomannans undergoing cleavage of galactose groups by specific enzyme action.

Depending on the size and interaction of the substituent groups, $[\eta]$ will also reflect changes in intrinsic chain flexibility or solvent quality. However, pendant galactose groups are linked 1–6 through a -CH_2–O- bridge, which possesses high conformational flexibility and is far removed spatially from the backbone in comparison to 1–2 or 1–3 linkage types. Little if any effect on the conformation of galactomannans from galactose side chains was found in one study of acetylated guar (Koleske & Kurath, 1964), and this was substantially confirmed in a recent study examining chain flexibility of several different galactomannans with varying galactose content (Picout et al., 2002). This analysis has again been repeated below on the SEC fraction data, and we have drawn identical conclusions.

From these arguments, the MHS prefactor can be considered to reflect intrinsic chain stiffness as well an artificial component reflecting average chain density. The bias from pendant groups on $[\eta]$ is substantial here because the galactose groups are as massive as the backbone mannose units; for many other types of substituted polysaccharides, the bias from chain density may not be registered at all. The apparent MHS constant $K_l\eta_l$ should therefore decrease with increasing galactose content. In relation to the MHS log–log plot, increasing galactose content forces two changes: a vertical, negative shifting due to galactose 'dead weight', and a horizontal, positive shifting to account for chain repeat mass M_o, which increases in proportion with DG. Note that the discussion is greatly simplified by adopting R_v, an extensive measure of coil size, rather than $[\eta]$; here it can be shown that R_v–N relationships for all galactomannans are essentially identical.

These arguments are consistent with the data in Figs. 6 and 11, and were also anticipated in previous studies. Doublier and Launey suggested the following empirical relationship, which incorporates galactose-dependent MHS coefficients:

$$[\eta] = 11.55 \times 10^{-4}[(1-x)M_w]^{0.98}$$

where $[\eta]$ has units ml/g, x is the mol% galactose (Doublier & Launay, 1981). This relationship has been used widely, despite the high exponent ($a = 0.98$), even though later studies have found exponents within the range expected for random coils (see referenced data in Picout and Ross-Murphy, 2007). Using the more accurate

Table 2
Weight-average molar mass of galactomannans reported in the literature.

Study	Galactomannan	M_w (kg/mol)	Method
Pollard et al. (2008)	Carob	1105	SEC-LALS/RI/Visc.
Cunha, de Paula, and Feitosa (2007)	Guar	4500 (M_{peak})	SEC-RI (Conv. Cal.)
Funami et al. (2005)	Guar	3460	SEC-MALS/RI
Brummer et al. (2003)	Carob	1198	SEC-RALS/RI/Visc.
	Guar	1304	
	Fenugreek	1418	
Daas et al. (2002)	Carob	429–632	SEC-RALS/RI/UV
	Tara	582–640	
	Guar	50–1602	
Simonet et al. (2002)	Guar	2170	Light scattering
Cheng, Brown, and Prud'homme (2002a)	Guar	1935	SEC-RI (Uni. Cal.)
Cheng, Brown, and Prud'homme (2002b)	Guar	1980	SEC-RI (Uni. Cal.)
Picout et al. (2002)	Carob	1050	SE-MALS/RI
	Tara	2250	
Laguna et al. (2003)	Guar	4200 ± 900 (H_2O)	SEC-MALS/RI
	Guar	2200 ± 200 (0.5 M K_2SO_4)	
Picout et al. (2001)	Guar	1870	SEC-MALS/RI
Wientjes, Duits, Jongschaap, and Mellema (2000)	Guar	1400	SEC-MALS/RI
Antonov et al. (1999)	Guar	350	Light scattering
Beer et al. (1999)	Guar	1600	SEC-RALS/RI/Visc.
Richardson et al. (1998)	Carob	812 (H_2O)	SEC-MALS/RI
	Carob	370 (0.05 M Phosph. Buffer)	
	Guar	1220 (0.05 M Phosph. Buffer)	
Jumel, Harding, anc Mitchell (1996)	Guar	2700	SEC-MALS/RI
Frollini, Reed, Milas, Rinaudo (1995)	Guar	1270	SEC-MALS/RI/Visc.
Gaisford, Harding, Mitchell, and Bradley (1986)	Carob	320-325	Sedimentation
Vijayendran and Bone (1984)	Guar	800	
	Guar	2200	SEC-LALS/RI
		2100	Light scattering
Robinson et al. (1982)	Guar	1650	Light scattering
Doublier and Launay (1981)	Guar	1700	Light scattering
Barth and Smith (1981)	Guar	>2000	SEC/RI

data of Robinson et al. (1982) from guar galactomannan, Fernandes et al. (1991) later suggested:

$$[\eta] = 3.84 \times 10^{-4}[M^{a}_{\text{backbone}}(1-x)^{-a}N_v]^{0.723}$$

Here M_{backbone} refers to the molar mass of mannose, the backbone unit, 162 g/mol, and N_v is viscosity-average chain length. Unfortunately, it does not appear that calibrations were ever carried out to test either of these models with different galactomannan types, even though they seem to be widely used for estimating M_w of galactomannans with variable DG (e.g., most recently, Cerqueira et al., 2009). Our model is very similar to that proposed by Fernandes et al. (1991) but using the chain repeat mass M_o instead of DG.

In a recent review it was argued that differences in the MHS plots for carob, tara, and guar galactomannans were not significant, within typical error (Picout and Ross-Murphy, 2007). Here, the shifts observed are well outside measurement error, and consistent with the arguments above. One consequence of variable prefactors in the MHS relationship is that using guar $K_{[\eta]}$ and a pairs as a proxy for other galactomannans is not recommended. Using this approach here gives M_w estimates for carob of ca. 2 million g/mol, *double* that obtained by the absolute LALS measurement. Clearly it is necessary to use MHS coefficients only for polymers of the same chain composition from which the calibration curves were developed, if accurate M_w estimates are desired. Alternatively, the model discussed above will provide a useful estimate.

3.7. Chain flexibility, persistence length, and unperturbed parameter K_Θ

Chain flexibility and unperturbed dimensions were determined using SEC fraction data closely following the analysis presented in previous studies (Picout et al., 2002; Robinson et al., 1982). The BSF plot is shown in Fig. 12 for those galactomannans whose DG has been determined previously. In the given form $[\eta]/M^{0.5}$ vs. $M^{0.5}$ the data are linear, indicating that within the limits of this approach, the intercept gives directly the unperturbed parameter K_Θ, from which chain stiffness C_∞ and persistence length L_p can be calculated.

In Fig. 13, all three parameters are plotted versus the degree of galactose substitution DG. K_Θ decreases with increasing DG in the same proportion as found for the MHS prefactor; here K_Θ has slope minus 1, which matches the depression expected from additional side group mass (i.e., mannose and galactose mass are equivalent). This finding is in complete agreement with the DG-dependence of the MHS prefactor $K_{[\eta]}$, and thus it is helpful to note again that K_Θ is an intensive quantity. For this reason, it may be more meaningful to report K_Θ for the (hypothetical) unsubstituted mannan chain; here the intercept gives $K_\Theta = 1.5 \pm 0.1$ ml/g. L_p and C_∞ were independent of DG, and by extension do not depend greatly on the galactomannan's botanical subfamily ($L_p = 5.4 \pm 0.4$ nm, $C_\infty = 20.3 \pm 0.8$ (Caesalpinioideae); $L_p = 5.5 \pm 0.2$ nm, $C_\infty = 19.9 \pm 1.3$ (Faboideae)).

Therefore, we conclude the intrinsic flexibility of the glycosidic bond of galactomannans is unaffected by galactose side groups, which further substantiates the conclusions reached by Picout et al. (2002) who reported nearly identical C_∞ for galactomannans derived from tara, guar, and carob. Nevertheless, our values for C_∞ and L_p are somewhat higher than reported in this and other studies ($C_\infty = 12.2 \pm 0.8$, Picout et al., 2002; $C_\infty \sim 12.6$, Robinson et al., 1982). Several reasons may account for this, but the most important is that we estimated K_Θ from native samples, whereas previous studies used lower-M fractions (Robinson et al. (1982) considered commercially-hydrolyzed guar, Picout et al. (2002) employed samples degraded by pressure–temperature treatment). We therefore repeated the analysis using the ultrasonicated fractions already available, and were able to reproduce similar values of C_∞ as well as L_p. This demonstrates merely that estimates of

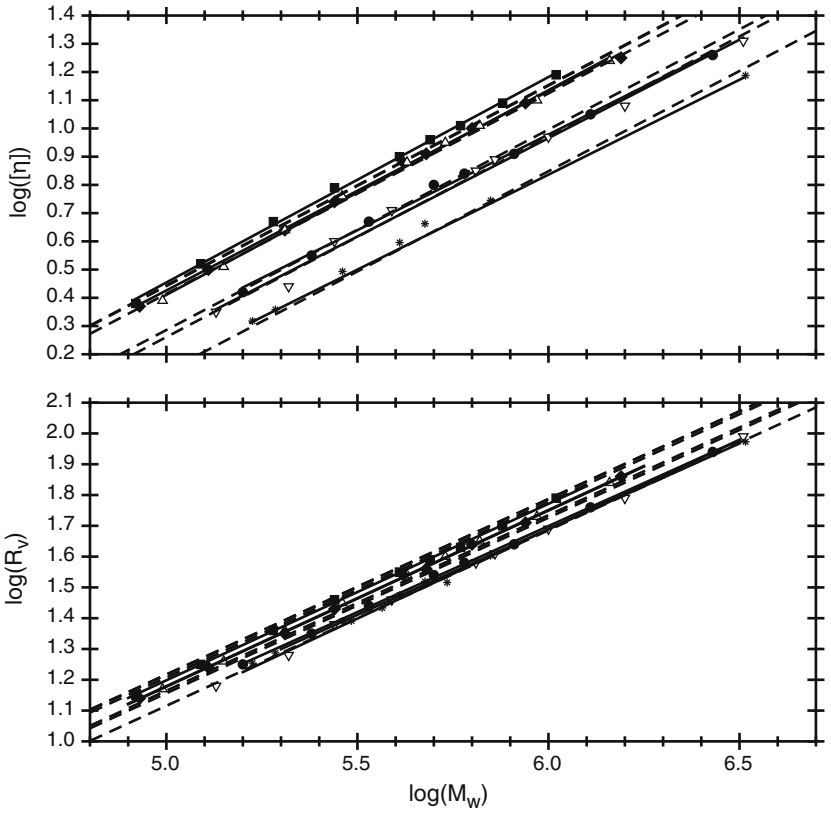

Fig. 11. Scaling of intrinsic viscosity $[\eta]$ (dl/g) and viscometric radius R_v (nm) for a series 6 galactomannans, obtained by ultrasonication. *C. siliqua* 'carob' (squares), *C. fistula* (up triangles), *C. spinosa* 'tara' (diamonds), *C. tetragonoloba* 'guar' (circles), *S. egyptica* (down triangles), and *T. foenum-graecum* 'fenugreek' (asterisk). Lines indicate linear fit, dashes indicate model prediction (see text).

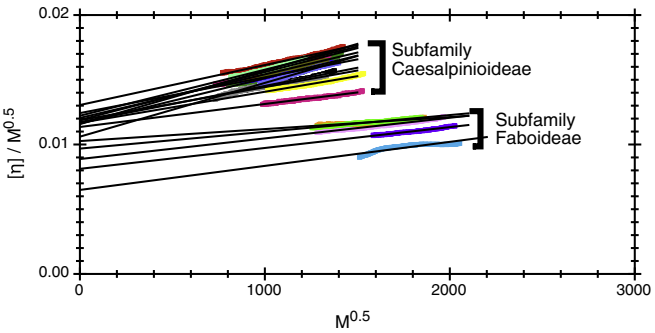

Fig. 12. Burchard–Stockmayer–Fixman (BSF) plots for estimation of unperturbed parameter K_Θ from the intercept.

chain flexibility with the BSF analysis depend sensitively on the molar mass fitting range, for these polysaccharides at any rate, much as the $[\eta]$–M relationships. A further interpretation of such effects is beyond the scope of this work.

4. Discussion

One consequence of the $[\eta]$–M relationships and observed patterns of variation in M_w is that intrinsic viscosities of native galactomannans are limited to a narrow range, despite substantial differences in their average molecular weight. For the samples investigated, M_w varies by about 3×, yet $[\eta]$ varies by less than a factor of 2. This appears to be a result of biosynthesis, which evidently yields galactomannans with increasingly longer average chain lengths as the characteristic value of DG increases, as shown by the correlation in Fig. 9. Thus, $[\eta]$ will increase with chain length in the manner expected by the Flory-Fox relation, but will decrease through the presence of galactose groups. Taking one example that may be relevant for applications, it can be shown by calculation that the fenugreek and guar samples have essentially identical chain length distributions. Therefore, fenugreek has ca. 30% higher M_w and correspondingly lower $[\eta]$ by about the same factor. Thus, for mass-efficient thickening, guar is the preferred option of these two galactomannans.

The correlation between M_w and DG for Caesalpinioideae galactomannans is an interesting finding. We speculate it could reflect events occurring on the molecular level during or after primary biosynthesis. One possible interpretation is that the chain length is regulated differently for these two botanical subfamilies. Chain growth during biosynthesis is known to involve the joint action of only two enzymes, one catalyzing chain growth (addition of mannose units to the backbone) and one catalyzing addition of galactose units to growing chains. Both mechanisms involve a condensation reaction of sugar precursors UDP-galactose and GDP-mannose (Reid, Edwards, Gidley, & Clark, 1995). Although chain termination mechanisms appear to be unknown, the correlation in Fig. 7 suggests that the latter enzyme may be involved in chain length regulation or chain termination for biosynthesis in Caesalpinioideae legumes, but not in Faboideae legumes.

It is also possible that the correlation represents modifications to the chain structure after biosynthesis has been completed. It has been found that DG of guar and fenugreek galactomannans is controlled during primary biosynthesis and is unchanged on extraction from mature seeds, whereas in *Senna occidentalis* α-galactosidase enzymes were found to be active during late galactomannan deposition, causing DG to drop (Edwards, Scott, Gidley, & Reid, 1992). Thus, it is possible that the correlation represents in-

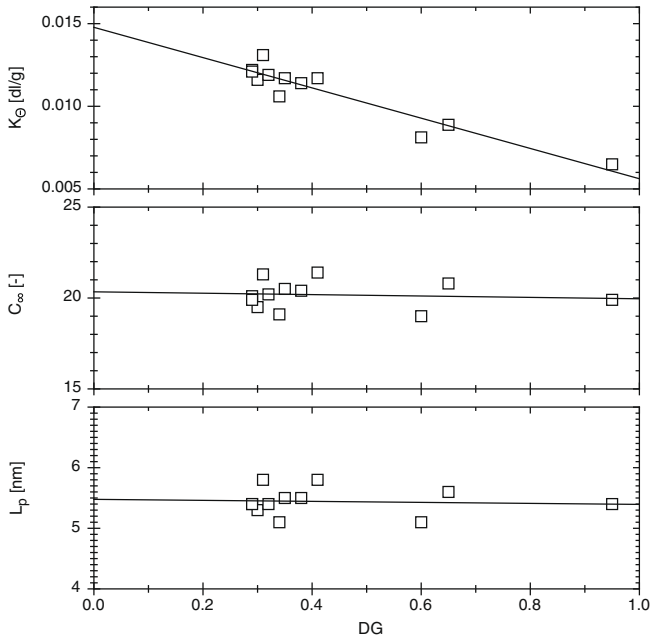

Fig. 13. Influence of galactose groups on unperturbed parameter K_Θ, Flory chain stiffness ratio C_∞, and chain persistence length L_p of galactomannans. For the hypothetical unsubstituted mannan, the intercept gives $K_{\Theta,\text{mannan}}$ = 1.5 ml/g.

stead the result of enzyme action, shifting DG to lower values depending on their activity and duration after primary biosynthesis. For this reason, we emphasize that our samples represent the molecular weights of galactomannans extracted from dry, viable seeds (through a defined extraction and solubilization method), not the primary biosynthetic product. Comparing galactomannans extracted during seed growth and maturation drying might be a way to test the validity of these ideas.

Buckeridge et al. (1995) reported that legumes within subfamily Caesalpinioideae had higher yields of galactomannan within their seeds, as well as lower DG than those derived from the Faboideae and Mimosoideae subfamilies. To these findings, we add that M_w of galactomannans from Caesalpinioideae seem to be much lower than those of Faboideae, albeit this is based on a smaller sampling of seeds. It also appears also that M_w and DG are correlated for galactomannans from this subfamily, though it is not clear whether this represents biosynthetic control or subsequent in vivo enzymatic action.

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References

Andrade, C. T., Azero, E. G., Luciano, L., & Gonçalves, M. P. (1999). Solution properties of the galactomannans from the seeds of *Caesalpinia pulcherrima* and *Cassia javanica*: Comparison with locust bean gum. *International Journal of Biological Macromolecules, 26*, 181–185.

Antonov, Y., Lefebvre, J., & Doublier, J. L. (1999). On the one-phase state of aqueous protein-uncharged polymer systems: Casein-guar gum system. *Journal of Applied Polymer Science, 71*, 471–482.

Armstrong, J. K., Wenby, R. B., Meiselman, H. J., & Fisher, T. C. (2004). The hydrodynamic radii of macromolecules and their effect on red blood cell aggregation. *Biophysical Journal, 87*, 4259–4270.

Bhattacharyya, S., Das, A. K., Banerji, N., & Farooqi, M. I. H. (1983). *A water-soluble galactomannan from Sesbania aegyptica, 22*, 161–164.

Barth, H. G., & Smith, D. A. (1981). High-performance size exclusion chromatography of guar gum. *Journal of Chromatography, 206*, 410–415.

Beer, M. U., Wood, P. J., & Weisz, J. (1999). A simple and rapid method for evaluation of Mark–Houwink–Sakurada constants of linear random coil polysaccharides using molecular weight and intrinsic viscosity determined by high performance size exclusion chromatography: Application to guar galactomannan. *Carbohydrate Polymers, 39*, 377–380.

Brummer, Y., Cui, W., & Wang, Q. (2003). Extraction, purification, and physicochemical characterization of fenugreek gum. *Food Hydrocolloids, 17*, 229–236.

Buckeridge, M. S., Panegassi, V. R., Rocha, D. C., & Dietrich, S. M. C. (1995). Seed galactomannan in the classification and evolution of the leguminosae. *Phytochemistry, 38*, 871–875.

Burke, M., Park, J., Srinivasarao, M., & Khan, S. (2005). A novel enzymatic technique for limiting drug mobility in a hydrogel matrix. *Journal of Controlled Release, 104*, 141–153.

Cerqueira, M., Pinheiro, A., Souza, B., Lima, Á., Ribeiro, C., Miranca, C., et al. (2009). Extraction, purification, and characterization of galactomannans from non-traditional sources. *Carbohydrate Polymers, 75*, 408–414.

Cheng, Y., Brown, K. M., & Prud'homme, R. K. (2002a). Characterization and intermolecular interactions of hydroxypropyl guar solutions. *Biomacromolecules, 3*, 456–461.

Cheng, Y., Brown, K. M., & Prud'homme, R. K. (2002b). Preparation and characterization of molecular weight fractions of guar galactomannans using acid and enzymatic hydrolysis. *International Journal of Biological Macromolecules, 31*, 29–35.

Cunha, P. L. R., de Paula, R. C. M., & Feitosa, J. P. A. (2007). Purification of guar gum for biological applications. *International Journal of Biological Macromolecules, 41*, 324–331.

Daas, P., Grolle, K., Vliet, T., Schols, H., & de Jongh, H. (2002). Toward the recognition of structure–function relationships in galactomannans. *Journal of Agricultural and Food Chemistry, 50*, 4282–4289.

Doublier, J. L., & Launay, B. (1981). Rheology of galactomannan solutions: Comparative study of guar gum and locust bean gum. *Journal of Texture Studies, 12*, 151–172.

Edwards, M., Scott, C., Gidley, M., & Reid, J. S. G. (1992). Control of mannose/galactose ratio during galactomannan formation in developing legume seeds. *Planta, 187*, 67–74.

Egorov, A. V., Mestechkina, N. M., & Shcherbukhin, V. D. (2003). Determination of the primary and fine structures of a galactomannan from the seed of *Gleditsia Triacanthos* f. inermis L. *Applied Biochemistry and Microbiology (Prikladnaya Biokhimiya i Mikrobiologiya), 39*, 398–402.

Fernandes, P. B., Gonçalves, M. P., & Doublier, J. L. (1991). A rheological characterization of kappa-carrageenan/galactomannan mixed gels: A comparison of locust bean gum samples. *Carbohydrate Polymers, 16*, 253–274.

Flory, P. (1936). Molecular size distribution in linear condensation polymers. *Journal of the American Chemical Society, 58*, 1877.

Frollini, E., Reed, W. F., Milas, M., & Rinaudo, M. (1995). Polyelectrolytes from polysaccharides: Selective oxidation of guar gum – a revisited reaction. *Carbohydrate Polymers, 27*, 129–135.

Funami, T., Kataoka, Y., Omoto, T., Goto, Y., Asai, I., & Nishinari, K. (2005). Food hydrocolloids control the gelatinization and retrogradation behavior of starch. 2a. Functions of guar gums with different molecular weights on the gelatinization behavior of corn starch. *Food Hydrocolloids, 19*, 15–24.

Gaisford, S., Harding, S., Mitchell, J., & Bradley, T. (1986). A comparison between the hot and cold water soluble fractions of two locust bean gum samples. *Carbohydrate Polymers, 6*, 423–442.

Gidley, M. J., & Reid, J. S. G. (1995). Galactomannans and other cell wall storage polysaccharides in seeds. In A. M. Stephen (Ed.), *Food polysaccharides and their applications*. New York: Marcel Dekker.

Joshi, H., & Kapoor, V. P. (2003). *Cassia grandis* Linn. f. seed galactomannan: Structural and crystallographical studies. *Carbohydrate Research, 338*, 1907–1912.

Jumel, K., Harding, S. E., & Mitchell, J. R. (1996). Effect of gamma irradiation on the macromolecular integrity of guar gum. *Carbohydrate Research, 282*, 223–236.

Kapoor, V. P. (1994). Rheological properties of seed galactomannan from *Cassia nodosa* buch-hem. *Carbohydrate Polymers, 25*, 79–84.

Kapoor, V. P., Taravel, F. R., Joseleau, J.-P., Milas, M., Chanzy, H., & Rinaudo, M. (1998). *Cassia spectabilis* DC seed galactomannan: Structural, crystallographical and rheological studies. *Carbohydrate Research, 306*, 231–241.

Koleske, J., & Kurath, S. (1964). Configuration and hydrodynamic properties of fully acetylated guaran. *Journal of Polymer Science A, 2*, 4123–4149.

Laguna, M. T. R., Tarazona, M. P., & Saiz, E. (2003). The use of molecular dynamics for the study of solution properties of guar gum. *Journal of Chemical Physics, 119*, 1148–1156.

Lal, J., & Gupta, P. (1972). Galactomannan from the seeds of *Cassia fistula*. *Planta Medica, 22*, 71–77.

McCleary, B., Amado, R., Waibel, R., & Neukom, H. (1981). Effect of galactose content on the solution and interaction properties of guar and carob galactomannans. *Carbohydrate Research, 92*, 269–285.

Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., & Rees, D. A. (1981). Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydrate Polymers, 1*, 5–21.

de Noy, A. E. J., Besemer, A. C., & van Bekkum, H. (1996). TEMPO-mediated oxidation of pullulan and influence of ionic strength and linear charge density on the dimensions of the obtained polyelectrolyte chains. *Macromolecules, 29*, 6541–6547.

Peebles, L. H. Jr., (1971). *Molecular weight distributions in polymers*. New York: Interscience. ISBN-10:0471677108, ISBN-13:9780471677109.
Picout, D. R., Ross-Murphy, S. B., Errington, N., & Harding, S. E. (2001). Pressure cell assisted solution characterization of polysaccharides. 1. Guar gum. *Biomacromolecules, 2*, 1301–1309.
Picout, D. R., Ross-Murphy, S. B., Jumel, K., & Harding, S. E. (2002). Pressure cell assisted solution characterization of polysaccharides. 2. Locust bean gum and tara gum. *Biomacromolecules, 3*, 761–767.
Picout, D. R., & Ross-Murphy, S. B. (2007). On the Mark–Houwink parameters for galactomannans. *Carbohydrate Polymers, 70*, 145–148.
Pollard, M. A., Kelly, R., Fischer, P. F., Windhab, E. J., Eder, B., & Amadó, R. (2008). Investigation of molecular weight distribution of LBG galactomannans for flours prepared from individual seeds, mixtures, and commercial samples. *Food Hydrocolloids, 22*, 1596.
Reid, J., Edwards, M., Gidley, M., & Clark, A. (1995). Enzyme specificity in galactomannan biosynthesis. *Planta, 195*, 489–495.
Robinson, G., Ross-Murphy, S. B., & Morris, E. R. (1982). Viscosity–molecular weight relationships, intrinsic chain flexibility, and dynamic solution properties of guar galactomannans. *Carbohydrate Research, 107*, 17–32.
Richardson, P. H., Willmer, J., & Foster, T. J. (1998). Dilute solution properties of guar and locust bean gum in sucrose solutions. *Food Hydrocolloids, 12*, 339–348.
Rechia, C., Sierakowski, M., Ganter, J., & Reicher, F. (1995). Polysaccharides from the seeds of senna multijuga. *International Journal of Biological Macromolecules, 17*, 409–412.
Reed, W. F. (1995). Data evaluation for unified multi-detector size exclusion chromatography – molar mass, viscosity, and radius of gyration distributions. *Macromolecular Chemistry and Physics, 196*, 1539.
Shcherbukhin, V., & Anulov, O. (1999). Legume seed galactomannans (review). *Applied Biochemistry and Microbiology, 35*, 229–244.
Schulz, G. V. (1939). Über die beziehung zwischen reaktionsgeswhwindigkeit und zusammensetzung des reaktionsproduktes bei macropolymerisationsvorgangen. *Zeitschrift für Physikalische Chemie, B43*, 25.
Shortt, D. (1993). Differential molecular-weight distributions in high-performance size-exclusion chromatography. *Journal of Liquid Chromatography, 16*, 3371.
Simonet, F., Garnier, C., & Doublier, J. L. (2002). Description of the thermodynamic compatibility of the guar-dextran aqueous two-phase system by light scattering. *Carbohydrate Polymers, 47*, 313–321.
Srivastava, M., & Kapoor, V. (2005). Seed galactomannans: An overview. *Chemistry and Biodiversity, 2*, 295–317.
Srivastava, H., Singh, P., & Subba Rao, P. (1968). A galactomannan from the seeds of *Sesbania grandiflora* pers. *Carbohydrate Research, 6*, 361–366.
Tewari, K., Singh, V., & Gupta, P. (2005). A non-ionic water soluble seed-gum from *Parkinsonia aculeata*. *Carbohydrate Polymers, 59*, 369–393.
Vijayendran, B. R., & Bone, T. (1984). Absolute molecular weight and molecular weight distribution of guar by size exclusion chromatography and low-angle laser light scattering. *Carbohydrate Polymers, 4*, 299–313.
Vold, I. M. N., Kristiansen, K. A., & Christensen, B. E. (2006). A study of the chain stiffness and extension of alginates, in vitro epimerized alginates, and periodate-oxidized alginates using size-exclusion chromatography combined with light scattering and viscosity detectors. *Biomacromolecules, 7*, 2136–2146.
Wientjes, R. H. W., Duits, M. H. G., Jongschaap, R. J. J., & Mellema, J. (2000). Linear rheology of guar gum solutions. *Macromolecules, 33*, 9594–9605.
Zimm, B. H. (1948). Apparatus and methods for measurement and interpretation of the angular variation of light scattering; preliminary results on polystyrene solutions. *Journal of Chemical Physics, 16*, 1099.